CLINICAL, MOLECULAR, AND EPIDEMIOLOGIC STUDIES OF XERODERMA PIGMENTOSUM AND RELATED DISORDERS OF DNA REPAIR

Kenneth H. Kraemer, M.D.
Chief, DNA Repair Section
Basic Research Laboratory
Center for Cancer Research
National Cancer Institute, Bethesda, MD

- CLINICAL FEATURES OF DNA REPAIR DISORDERS
- MOLECULAR ABNORMALITIES IN XERODERMA PIGMENTOSUM PATIENTS
- EPIDEMIOLOGICAL STUDIES OF XP GENE POLYMORPHISMS AND XP FAMILIES

- **CLINICAL FEATURES OF DNA REPAIR DISORDERS**
- **MOLECULAR ABNORMALITIES IN XERODERMA PIGMENTOSUM PATIENTS**
- **EPIDEMIOLOGICAL STUDIES OF XP GENE POLYMORPHISMS AND XP FAMILIES**

DNA REPAIR DISEASES

CLINICAL DISORDERS AND MOLECULAR DEFECTS

XERODERMA PIGMENTOSUM

XERODERMA PIGMENTOSUM WITH NEUROLOGICAL ABNORMALITIES

XERODERMA PIGMENTOSUM/ COCKAYNE SYNDROME COMPLEX

COFS SYNDROME

COCKAYNE SYNDROME

XP/TTD

TRICHOTHIODYSTROPHY

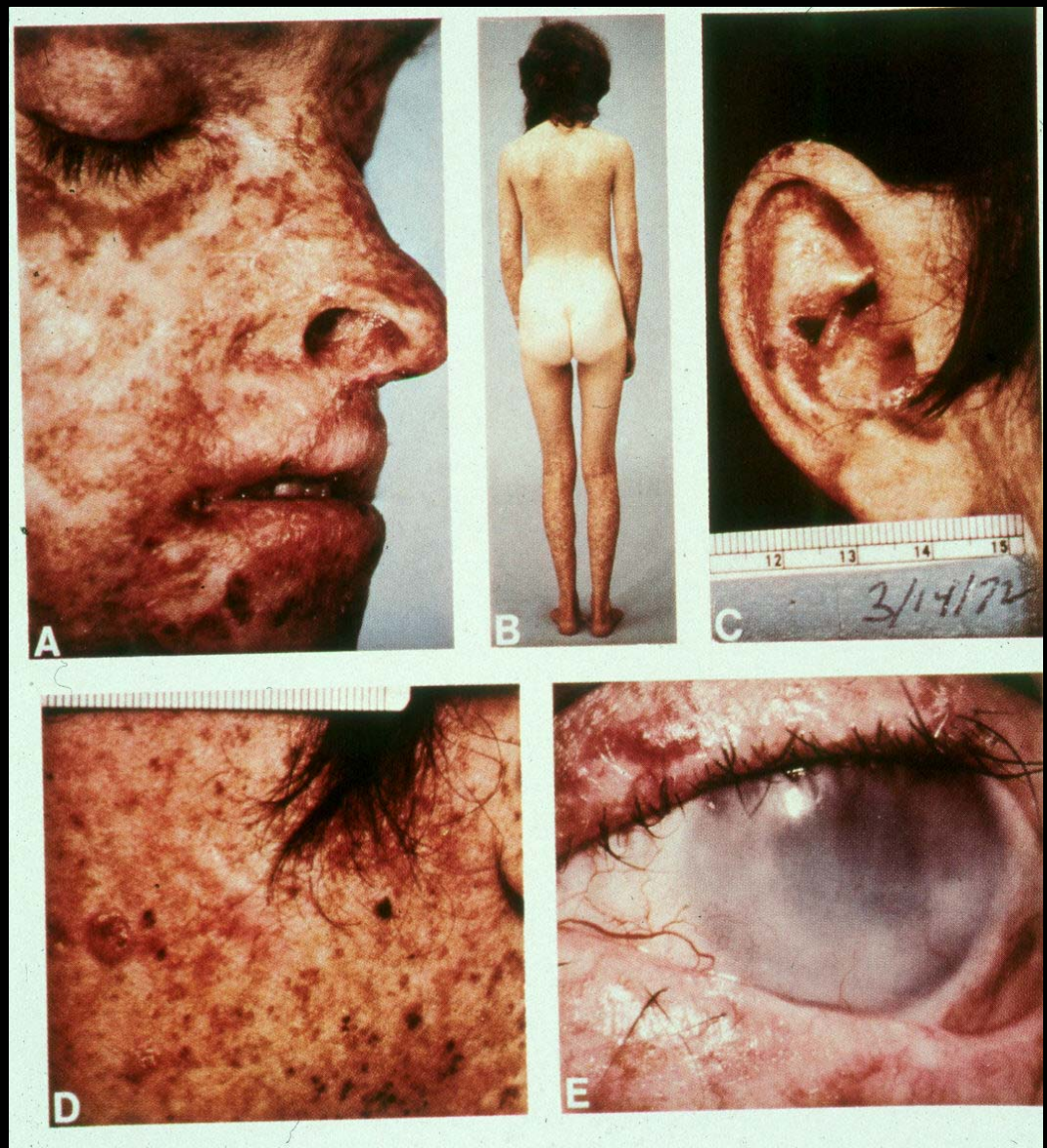
Kraemer Nature Genetics 36: 677- 8, 2004

MORITZ KAPOSI

First description of XP patients - 1870

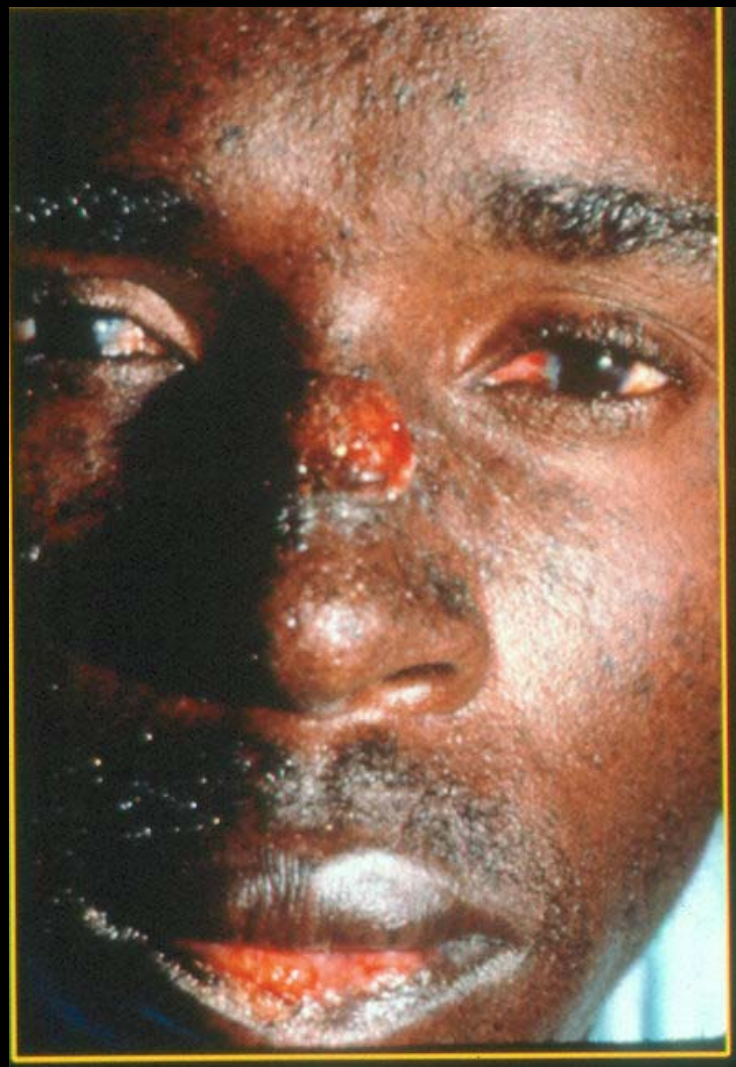
Moriz Kohn 1837 - 1902

XERODERMA PIGMENTOSUM CLINICAL FEATURES

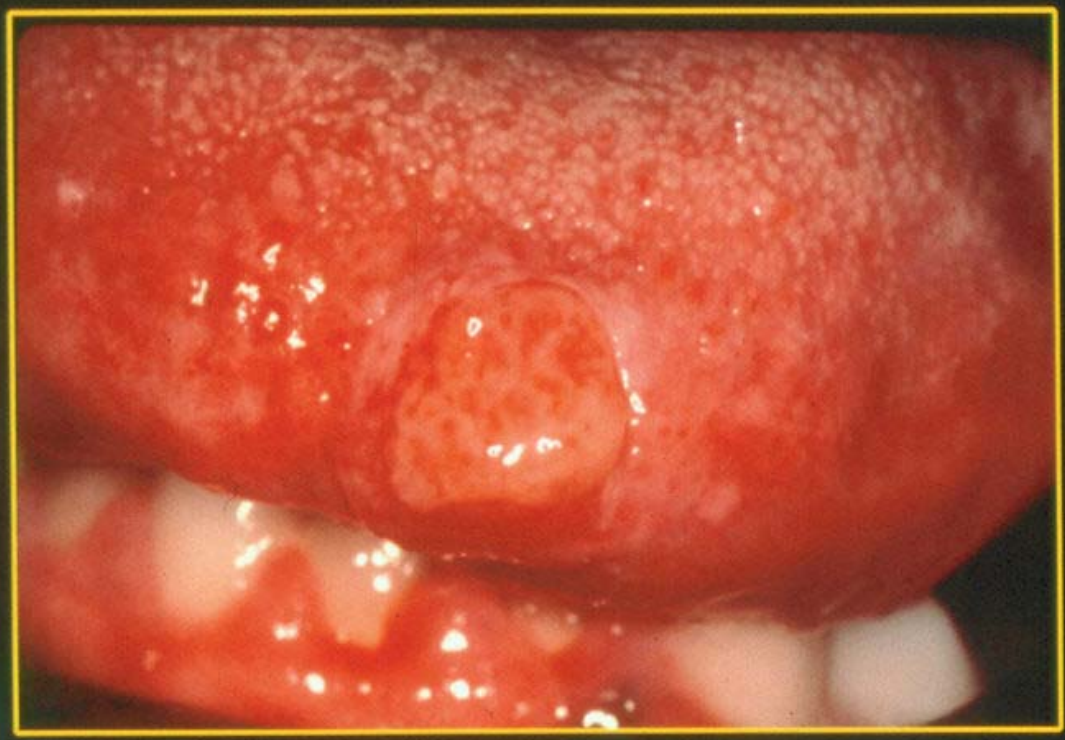


Van Steeg & Kraemer
Mol Med Today 5;
86-94, 1999

AFRICAN AMERICAN WITH XERODERMA PIGMENTOSUM



SCC FACE



SCC TONGUE

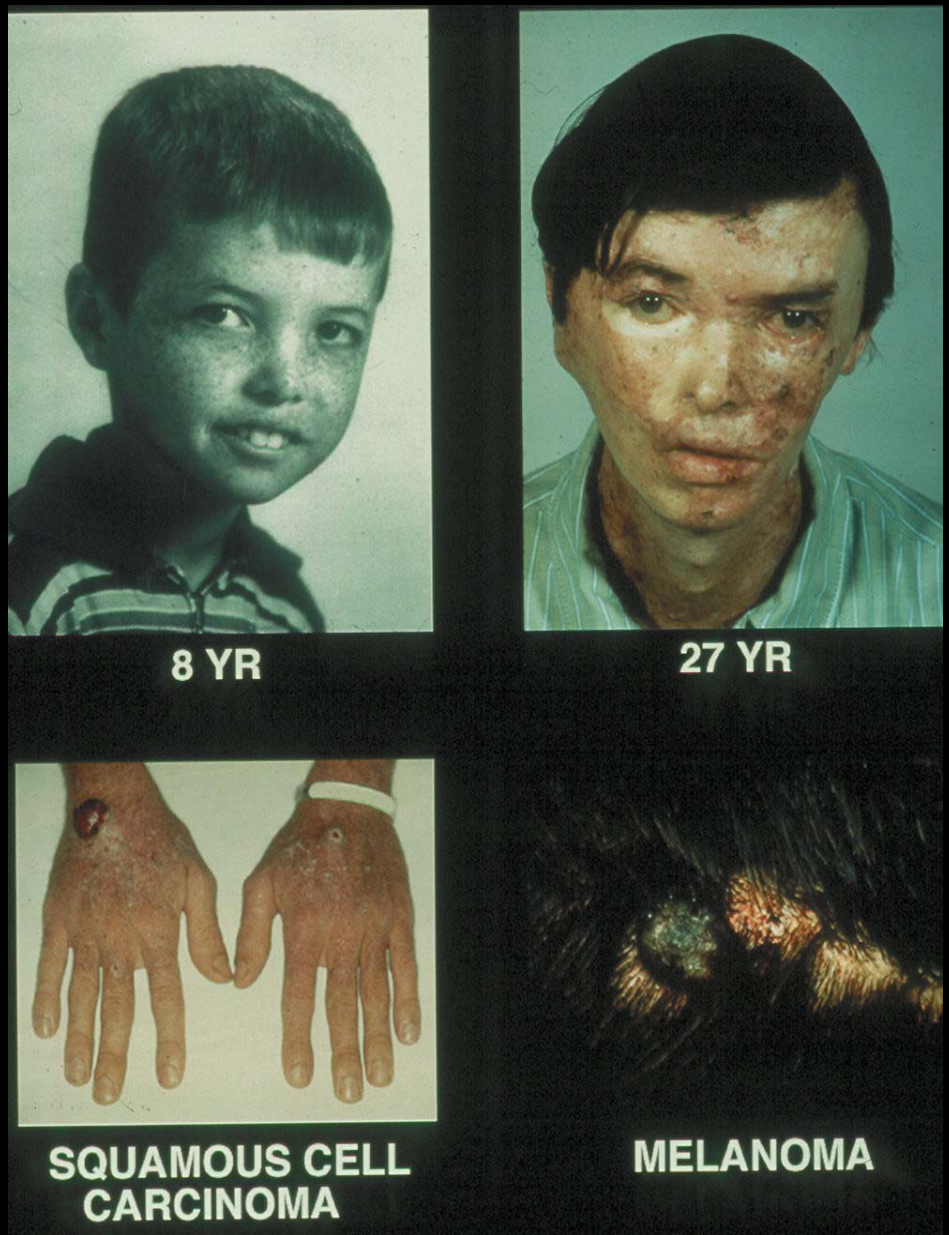
XERODERMA PIGMENTOSUM

Autosomal recessive
Clinical sun sensitivity, marked freckling
SKIN CANCERS (BCC, SCC, Melanoma)

Cellular UV hypersensitivity
Defective DNA repair
7 nucleotide excision repair complementation groups
(XPA, XPB, XPC, XPD, XPE, XPF, XPG)
VARIANT with normal NER – defective bypass polymerase

Chromosomes: 9q34 (A), 2q21 (B), 3p25.1 (C), 19q13.2 (D),
11p12-p11 (E), 16p13.3 (F), 13q33 (G), 6p21.1 (Variant)
Cloned genes *XPA*, XPB (*ERCC3*), *XPC*, XPD (*ERCC2*),
XPE (*DDB2*), XPF (*ERCC4*), XPG (*ERCC5*), Variant (*POLH*)

XERODERMA PIGMENTOSUM VARIANT - XP4BE



Annals Internal Med
80: 221-248, 1974

XP Features

- Equally distributed among males and females
- Ethnicity;

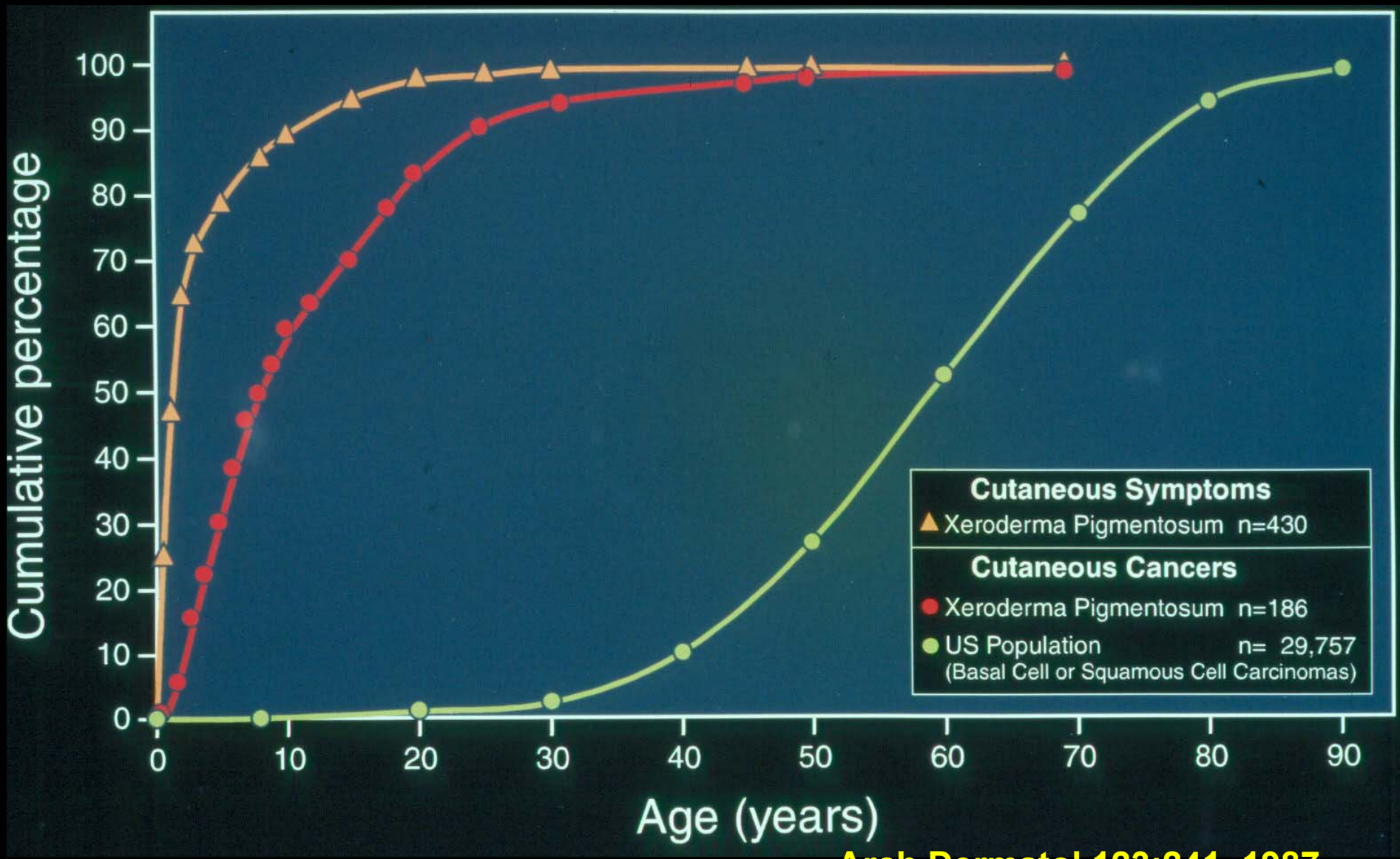
Occurance

  - Middle East
  - Europe
  - Japan
  - Africa
  - America

- Frequency: 1:100,000 in Japan
- Frequency: 1:1,000,000 in U.S.

- **Oldest patient: 85 yr**
- **Sun sensitivity or freckling (median age of onset): 1.5 yr**
- **Skin cancer (median age of onset): 8 yr**
- **97% of basal & squamous cells carcinoma occur on face, head, or neck**
- **65% of melanomas occur on face, head, or neck**
- **In the past, death occurred 30 yr earlier than in the US general pop.**
- **Sun protection may prolong life.**

EARLY AGE OF ONSET OF SKIN CANCER IN XP



Arch Dermatol 123:241, 1987

ELEVATED CANCER FREQUENCY IN XERODERMA PIGMENTOSUM

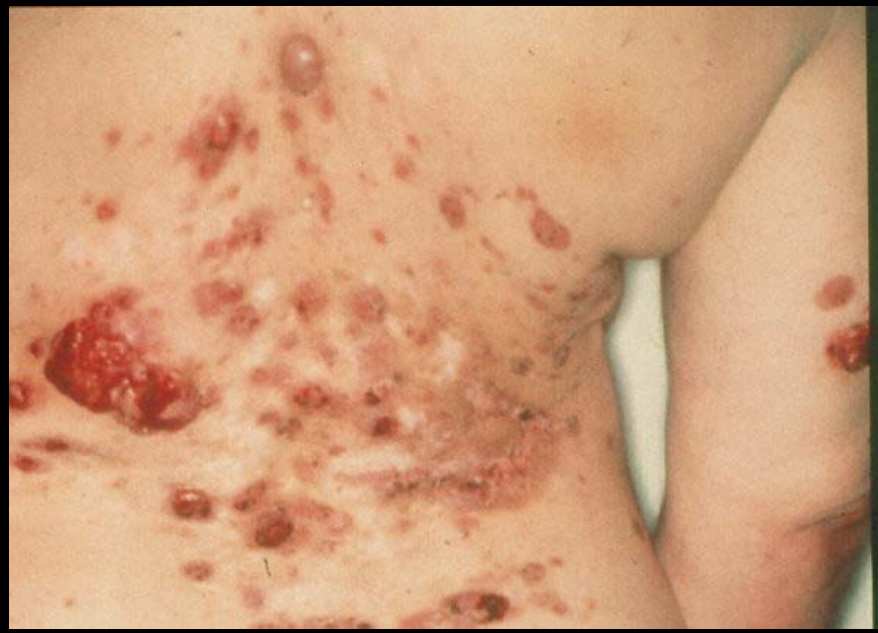
Table 1. Frequencies of Skin, Eye, Tongue, and Internal Cancers in Patients With Xeroderma Pigmentosum (XP) Compared With the US General Population

Cancer Sites	Age, y	No. of XP Patients	No. of XP Patients With Cancer		Ratio: Observed/Expected	95% C.I.‡
			Expected*	Observed†		
Sun-Exposed Sites						
Skin basal cell and squamous cell carcinomas	0-19	77	0.01	49	4900	3600-6500
	0-39	123	0.13	52	400	300-500
	All (0-62)	132	0.51	76	150	120-200
Skin melanomas	0-19	77	0.001	8	8000	3500-16 000
	0-39	123	0.022	14	600	350-1100
	All (0-62)	132	0.042	29	700	500-1000
Eye cancers	0-19	77	0.004	4	1000	300-2500
	0-39	123	0.007	5	700	200-1700
	All (0-62)	132	0.009	15	1700	900-2800
Tongue cancers	0-19	77	0.00003	3	100 000	21 000-300 000
	0-39	123	0.001	3	3000	600-9000
	All (0-62)	132	0.004	3	800	200-2000
Sun-Shielded Sites						
All internal cancers§	0-19	77	0.09	1	11.1	0.3-61.7
	0-39	123	0.36	3	8.3	1.7-24.3
	All (0-62)	132	0.81	4‖	4.9	1.3-12.7
Brain and other central nervous system	0-19	77	0.02	1	50	1.3-278
	0-39	123	0.05	2	40	4.8-144
	All (0-62)	132	0.06	2#	33	4.0-120

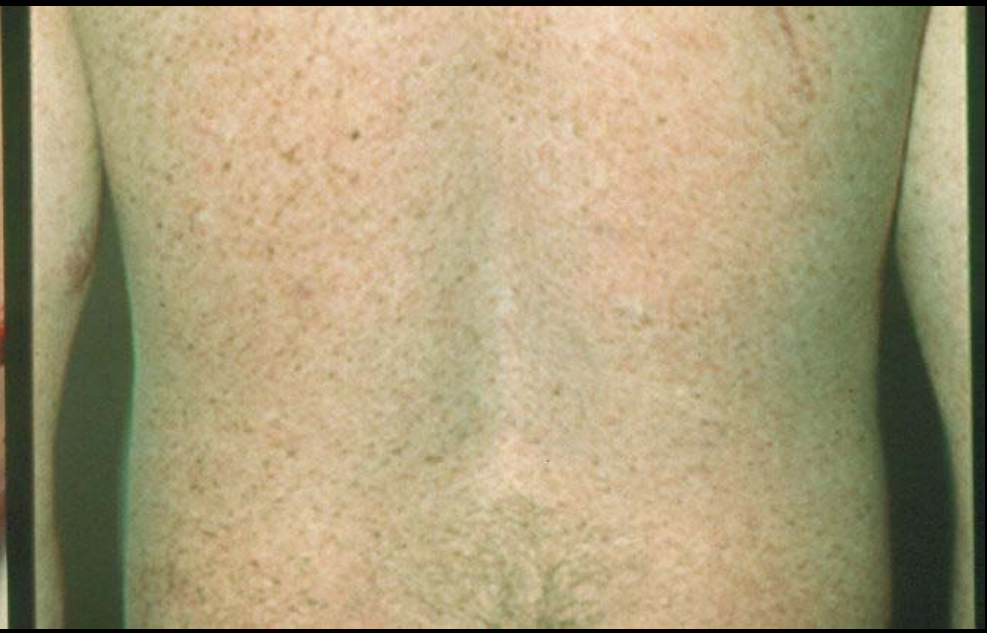
*Calculated from cumulative age-specific annual rates of basal cell and squamous cell carcinoma from Scotto et al[1]; others from Young et al.[8]
†Age at first neoplasm of indicated type.
‡95% confidence interval based on the Poisson distribution.[4]
§Including brain and other central nervous system but excluding melanoma of the eye, lip, and tongue.
‖Brain sarcoma, 16-year-old patient; spinal cord astrocytoma, 24-year-old patient; lung carcinoma, 34-year-old smoker; gastric cancer, 50-year-old patie
#Brain sarcoma, 16-year-old patient; spinal cord astrocytoma, 24-year-old patient (same cases listed above).

Arch Dermatol 130:1018, 1994

X-RAY HYPERSENSITIVITY IN Basal Cell Nevus Syndrome BUT NOT IN XP !



X-Ray Treatment of BCC in Basal Cell Nevus Syndrome
Multiple skin cancers in site of treatment

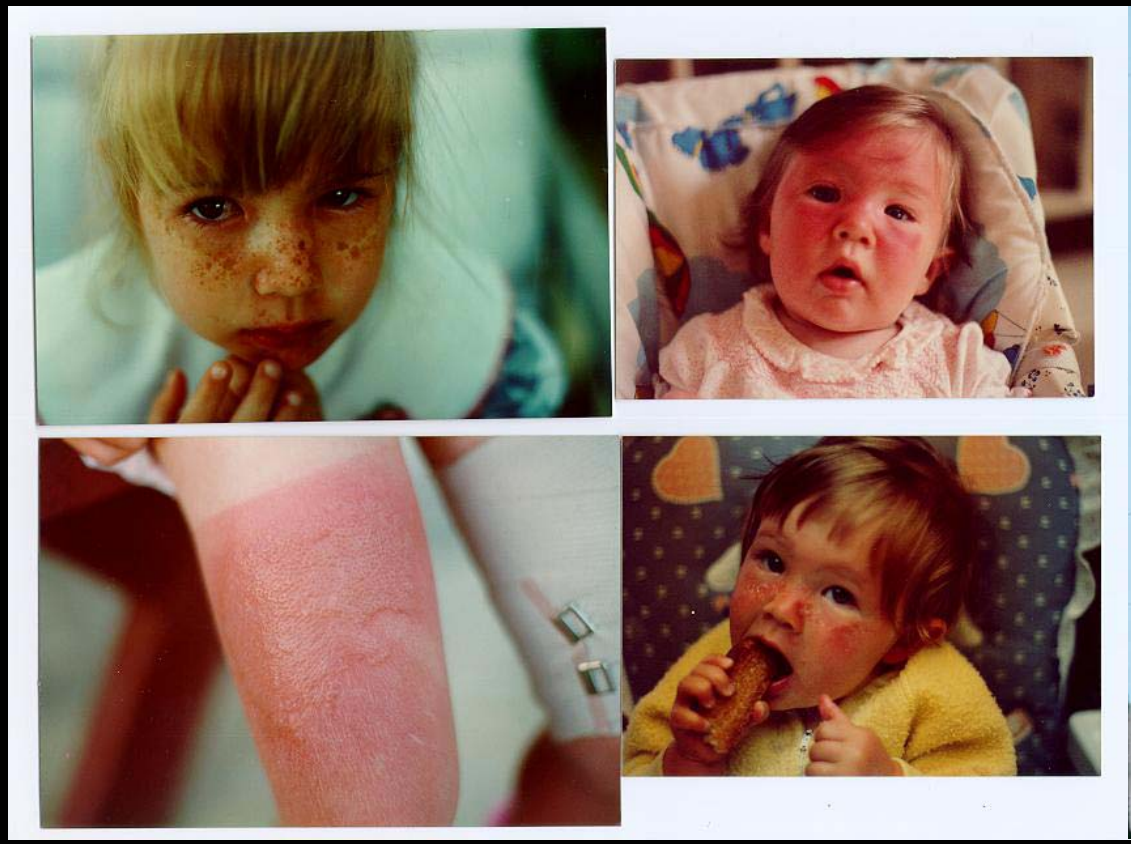


X-Ray Treatment of Spinal Cord Astrocytoma in XP
Normal response to treatment.

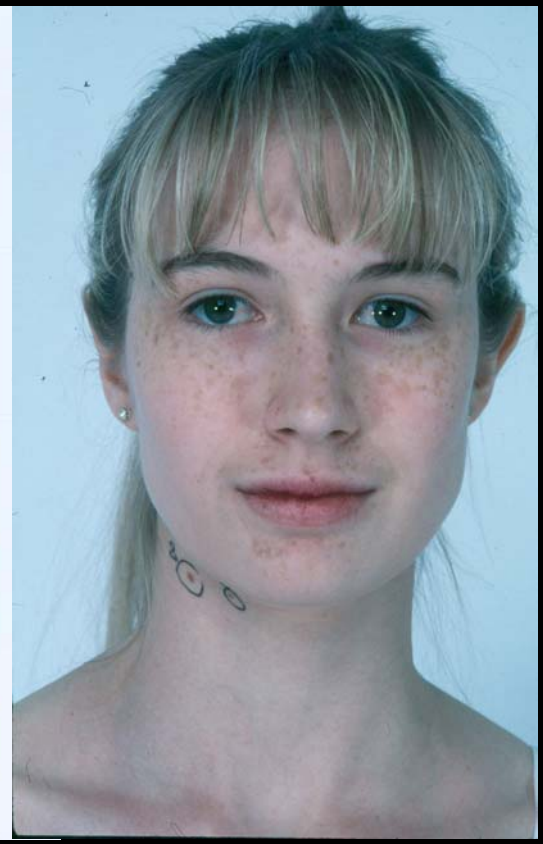
SUN PROTECTION WITH NASA SUIT IN XP

4 y/o

XERODERMA PIGMENTOSUM GROUP G



Marked sun sensitivity in childhood



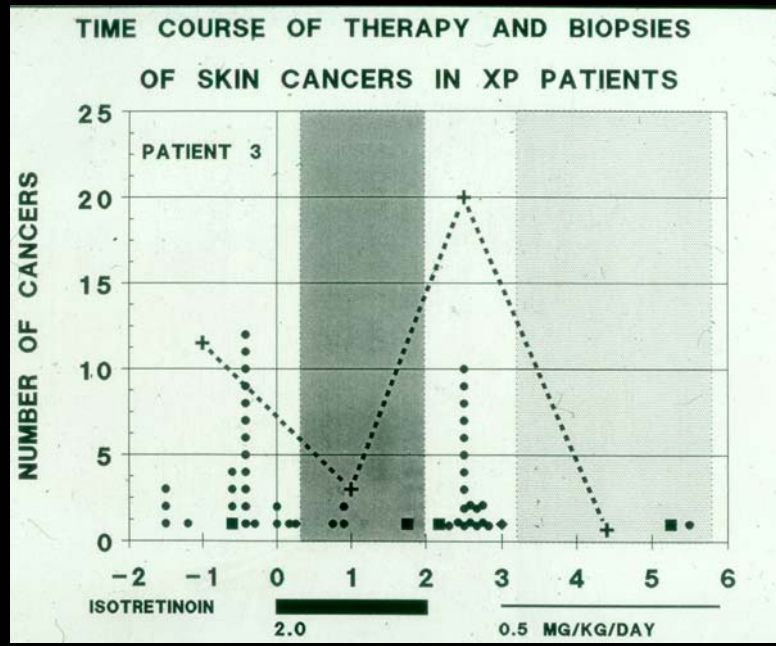
14 y/o – well protected
minimal skin changes

J Invest Dermatol 118: 972-82, 2002

ORAL ISOTRETINOIN PREVENTS NEW SKIN CANCERS IN XERODERMA PIGMENTOSUM

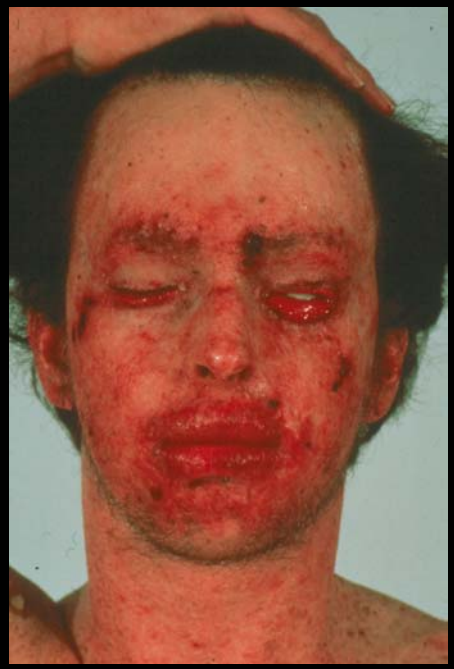
Table 1. Number of Skin Cancers in Patients with Xeroderma Pigmentosum before, during, and after Therapy with Oral Isotretinoin (2 mg per Kilogram per Day).

Patient	Age/Sex	Before Treatment* (2 Yr)	During Treatment* (2 Yr)	After Treatment† (12–14 Mo)
		number (number per year)		
1	19/F	43 (21.5)	3 (1.5)	18 (18.0)
2	12/F	37 (18.5)	4 (2.0)	29 (38.7)‡
3	17/M	23 (11.5)	6 (3.0)	20 (20.0)
4	39/M	10 (5.0)	3 (1.5)	4 (3.4)
5	10/M	8 (4.0)	9 (4.5)	10 (10.0)



Kraemer et al New Engl J Med 315: 1615, 1986

SIDE EFFECTS OF ORAL ISOTRETINOIN FOR XERODERMA PIGMENTOSUM



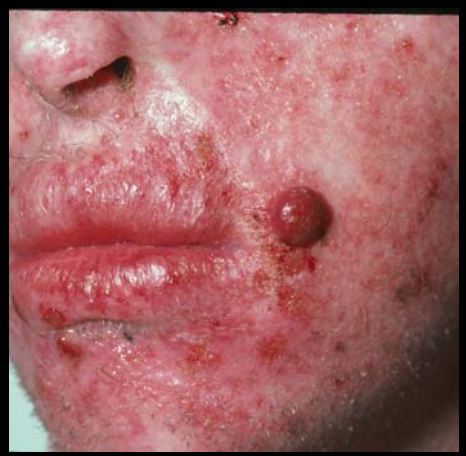
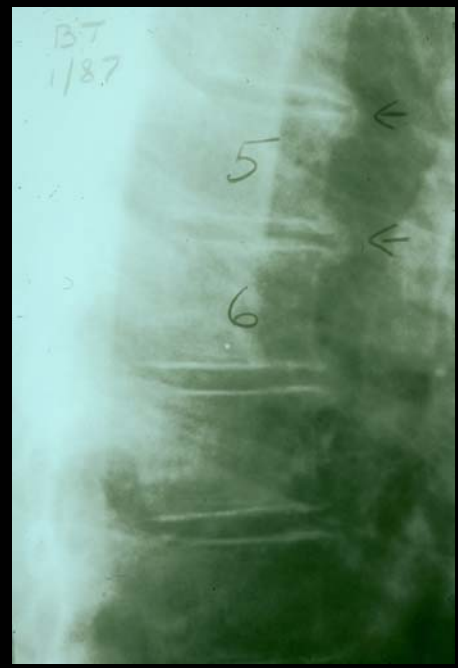


Table 2. Frequency of Side Effects Observed in Seven Patients with Xeroderma Pigmentosum during Treatment with Oral Isotretinoin (2 mg per Kilogram per Day).

Side Effect	No. of Patients Affected
Dry skin	7
Cheilitis	7
Blepharitis or Conjunctivitis	7
Lightening or disappearance of freckles	6
Increased serum triglycerides	6
Abnormal liver-function tests	4
Arthralgias	4
Staphylococcal infection (perioral)	3
Multiple pyogenic granulomas	2
Skeletal toxicity	2



Kraemer et al NEJM 315: 1615 (1986)

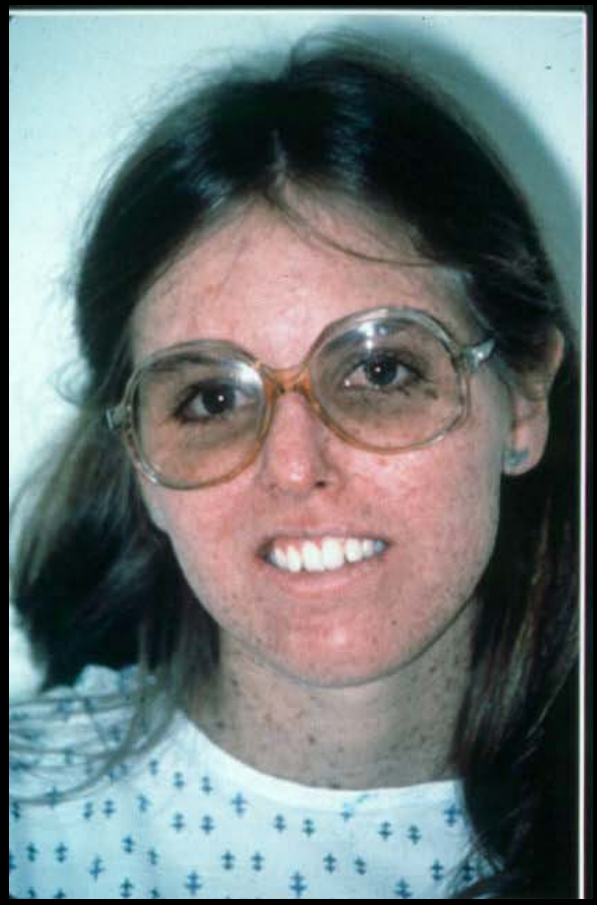
XERODERMA PIGMENTOSUM with NEUROLOGICAL ABNORMALITIES

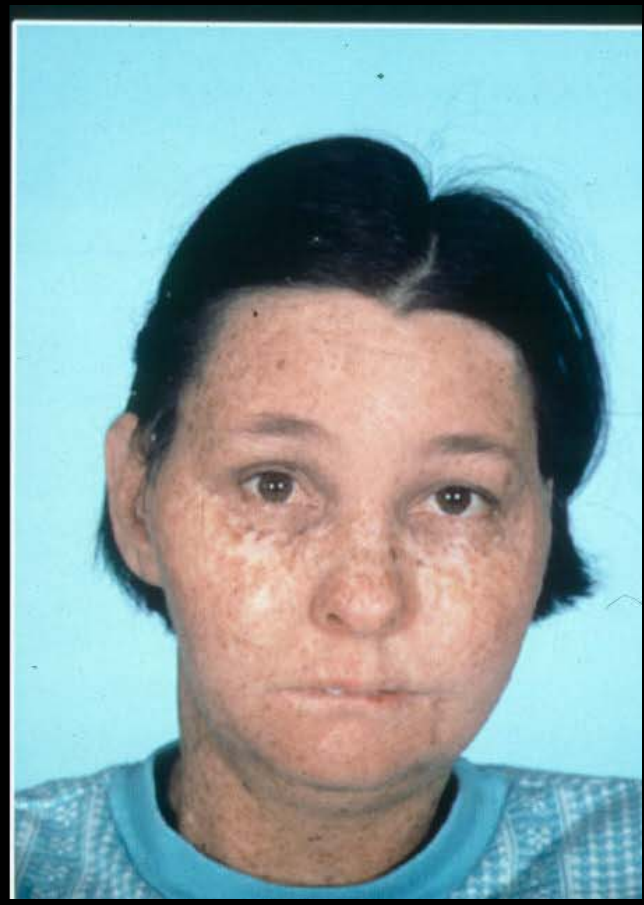
Autosomal recessive
Usually blistering on minimal sun exposure, Marked freckling
SKIN CANCERS (BCC, SCC, Melanoma)
Progressive neurological degeneration (20% of XP)
Primary neuronal degeneration
Progressive sensorineural deafness

Cellular UV hypersensitivity
Defective DNA repair
4+ nucleotide excision repair complementation groups
(XPA, XPB, XPD, XPG, rarely XPC)

Chromosomes: 9q34 (A), 2q21 (B), 3p25.1 (C), 19q13.2 (D), 13q33 (G)
Cloned genes *XPA*, XPB (*ERCC3*), *XPC*, XPD (*ERCC2*), XPG (*ERCC5*)

XERODERMA PIGMENTOSUM WITH NEUROLOGICAL ABNORMALITIES





XP12BE - XPA

XP11BE - XPB/CS

XP6BE - XPD

Annals Internal Med 80: 221-248, 1974

COCKAYNE SYNDROME

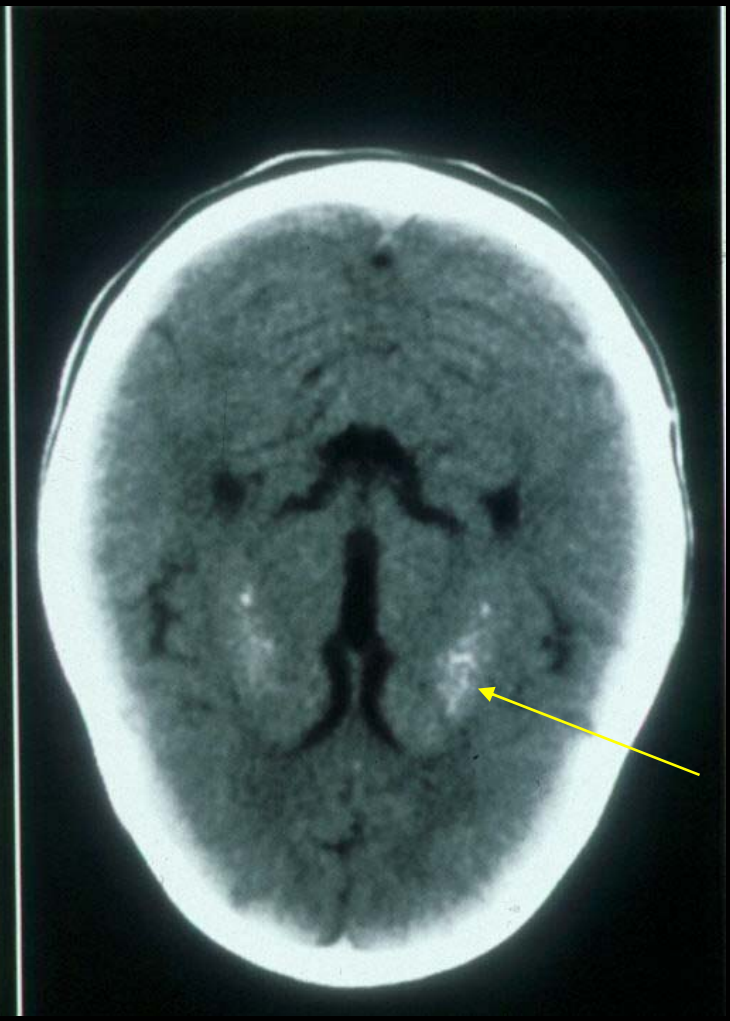
Autosomal recessive
Clinical sun sensitivity
Progressive neurological degeneration
Abnormal myelination of brain
Deafness, dwarfism, retinopathy

NO CANCER

Cellular UV hypersensitivity
Defective repair of actively transcribed genes
(defective TC-NER)
Defective repair of cyclobutane dimers
Normal repair of 6-4 UV photoproducts

2 Complementation groups (CSA, CSB)
Chromosome: 5 (CSA), 10q11 (CSB)
Cloned genes: CSA (*ERCC8*), CSB (*ERCC6*)

COCKAYNE SYNDROME



3 y/o

Calcification in
Basal Ganglia

XERODERMA PIGMENTOSUM / COCKAYNE SYNDROME COMPLEX

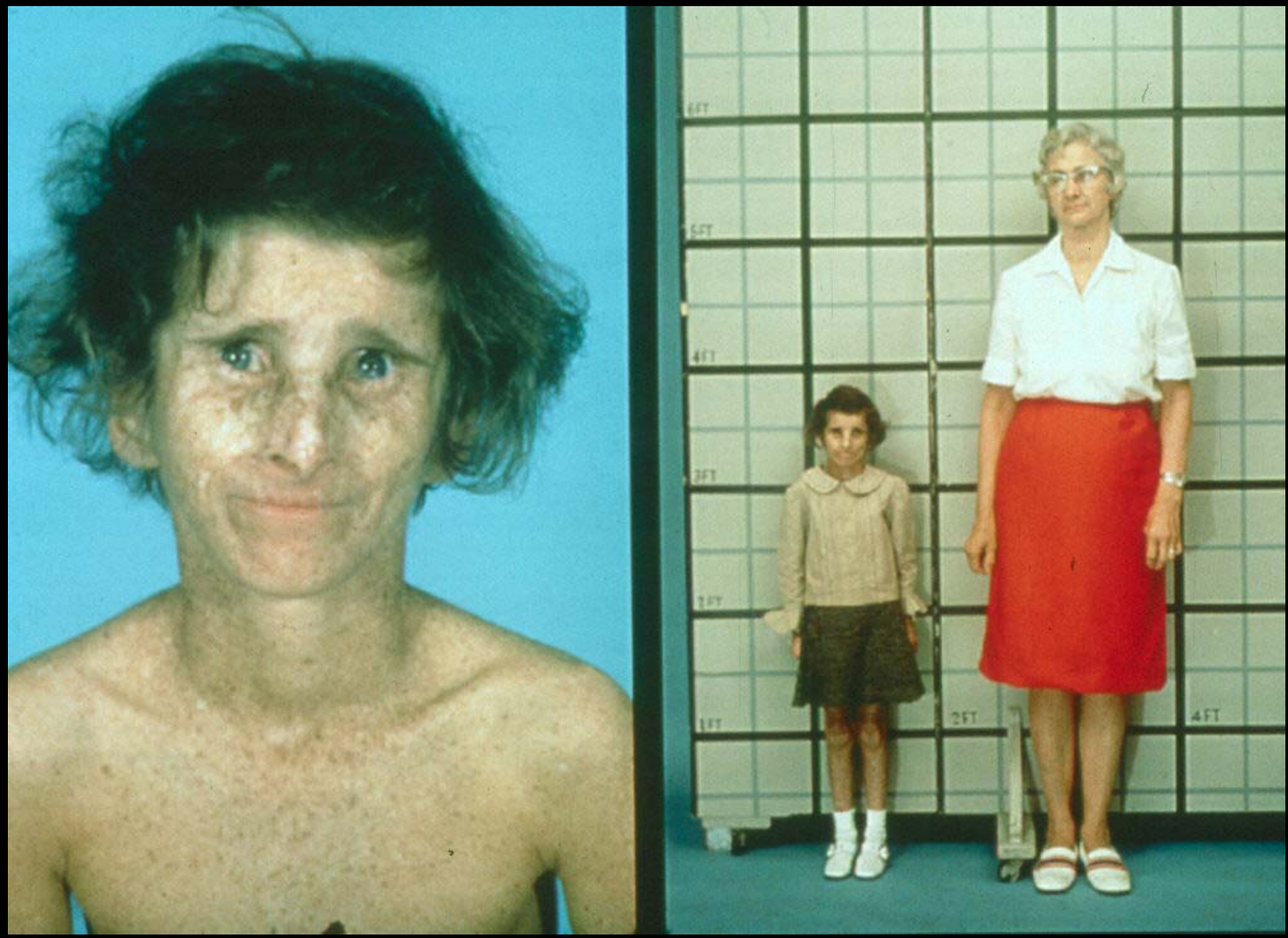
Neurological and somatic features of CS with Skin and cellular abnormalities of XP

SKIN CANCER

Cellular UV hypersensitivity
Defective DNA repair
3 XP complementation groups (XPB, XPD, XPG)

Chromosomes: 2q21 (B), 19q13.2 (D), 13q33 (G)
Cloned genes: XPB (*ERCC3*), XPD (*ERCC2*), XPG (*ERCC5*)

XERODERMA PIGMENTOSUM / COCKAYNE SYNDROME COMPLEX



XP/CS group B - XP11BE 28 y/o Mother

Annals Internal Med 80: 221-248, 1974

TRICHOTHIODYSTROPHY

Autosomal recessive
Photosensitive, Ichthyosis
Sulfur deficient Brittle hair
Intellectual impairment, Decreased fertility
Short stature (PIBIDS)

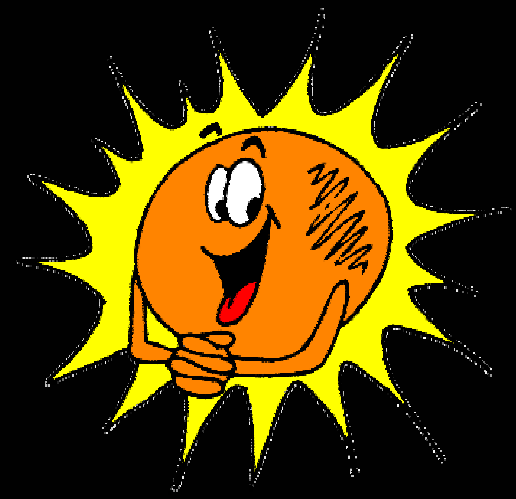
NO CANCER

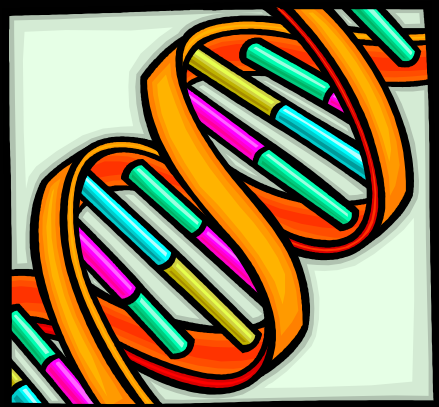
Cellular UV hypersensitivity
Defective DNA Repair
3 complementation groups (XPB, XPD, TTDA)

Chromosome: 2q21 (XPB), 19q13.2 (XPD)
Cloned genes: XPB (*ERCC3*), XPD (*ERCC2*), TTDA (*GTF2H5*)

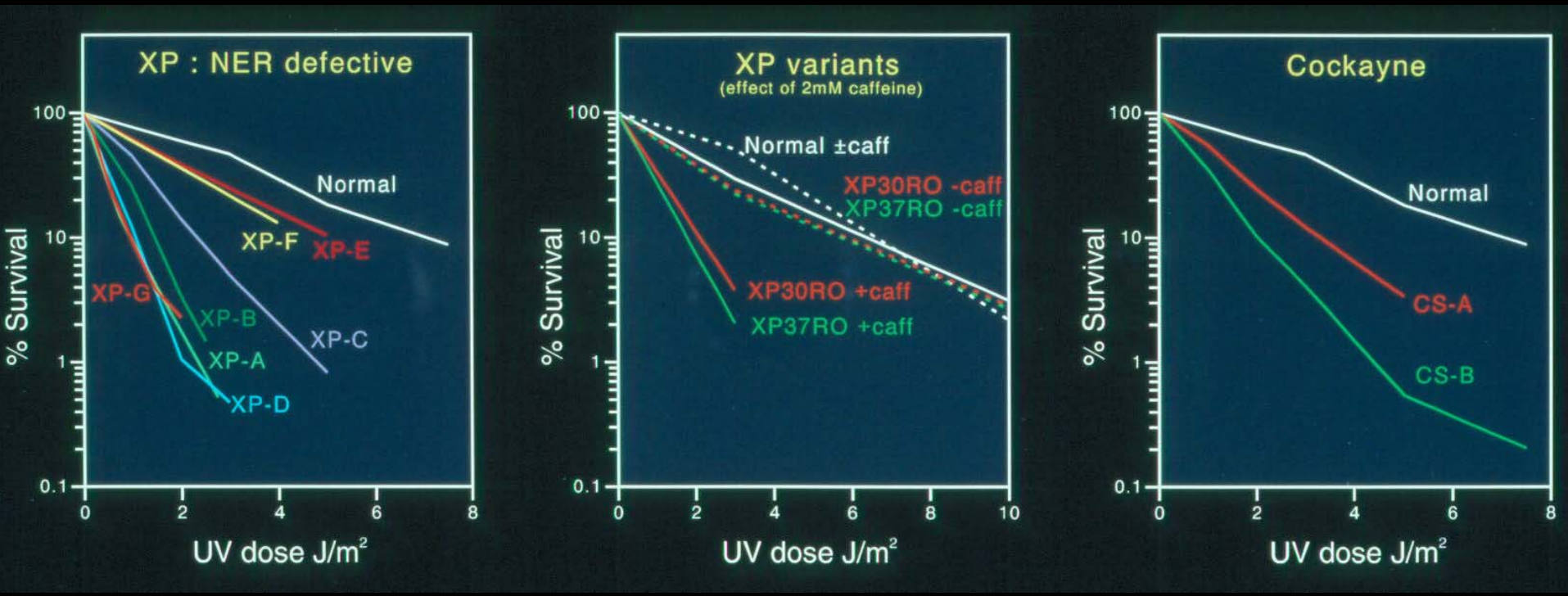
- CLINICAL FEATURES OF DNA REPAIR DISORDERS
- MOLECULAR ABNORMALITIES IN XERODERMA PIGMENTOSUM PATIENTS
- EPIDEMIOLOGICAL STUDIES OF XP GENE POLYMORPHISMS AND XP FAMILIES

DNA REPAIR – THE LIFEGUARD OF THE GENE POOL

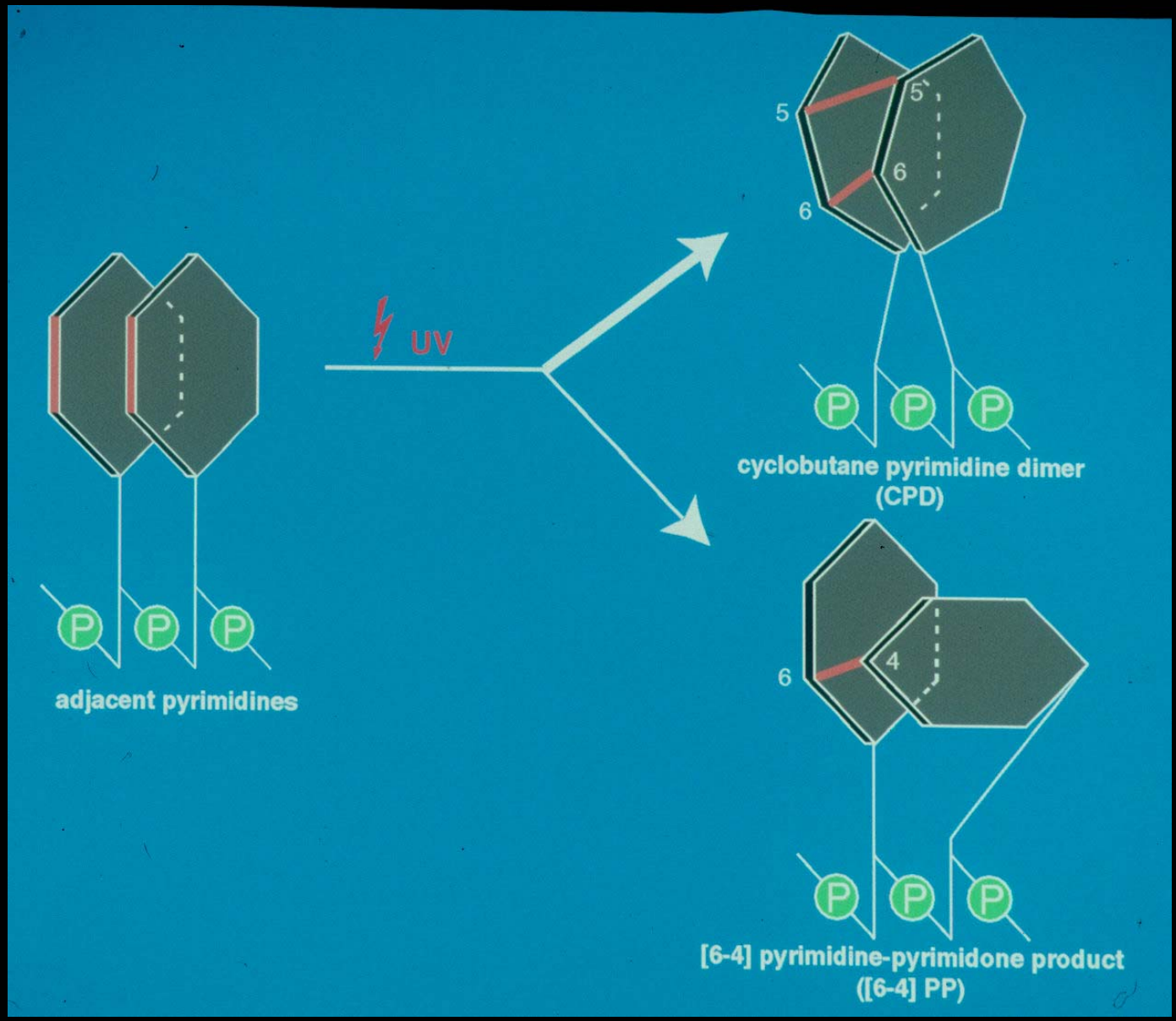




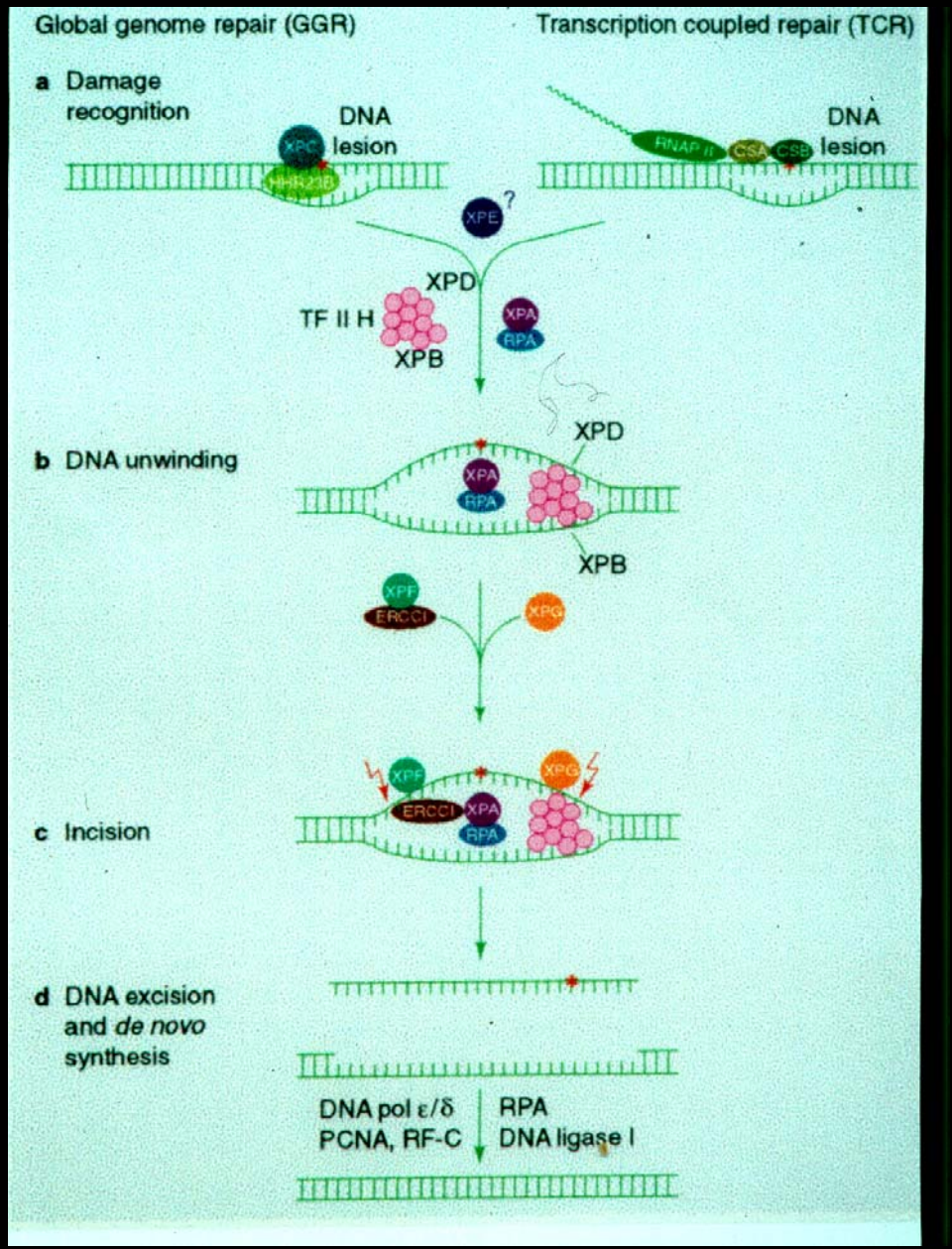
UV HYPERSENSITIVITY OF XP AND CS CELLS



UV PHOTOPRODUCTS



NUCLEOTIDE EXCISION REPAIR



Van Steeg & Kraemer
Mol Med Today 5;
86-94, 1999

DNA REPAIR DISEASES

CLINICAL DISORDERS AND MOLECULAR DEFECTS

XERODERMA PIGMENTOSUM

XPC

XPE

XPF

XPV

XPA

XERODERMA PIGMENTOSUM WITH NEUROLOGICAL ABNORMALITIES

XPG

XPD

XERODERMA PIGMENTOSUM/ COCKAYNE SYNDROME COMPLEX

XPB

XP/TTD

COFS SYNDROME

CSA

COCKAYNE SYNDROME

CSB

TRICHOTHIODYSTROPHY

TTD-A

Kraemer Nature Genetics 36: 677- 8, 2004

- CLINICAL FEATURES OF DNA REPAIR DISORDERS
- MOLECULAR ABNORMALITIES IN XERODERMA PIGMENTOSUM PATIENTS
- EPIDEMIOLOGICAL STUDIES OF XP GENE POLYMORPHISMS AND XP FAMILIES

DNA REPAIR GENES AND CANCER RISK

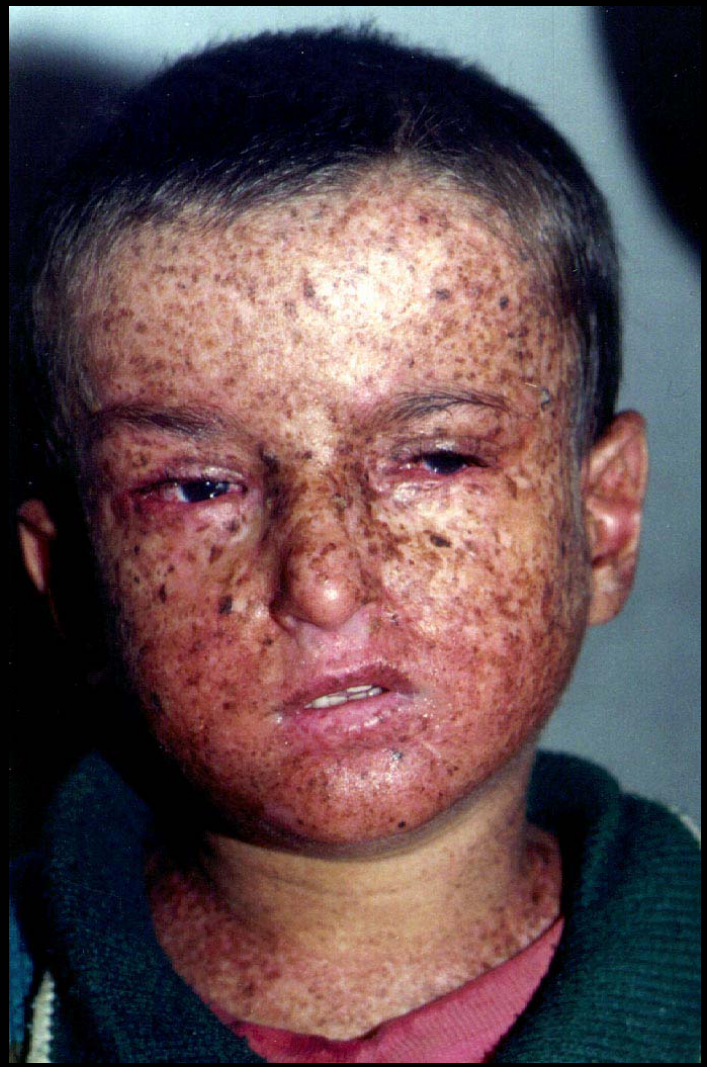
Disease gene homozygotes

- low frequency

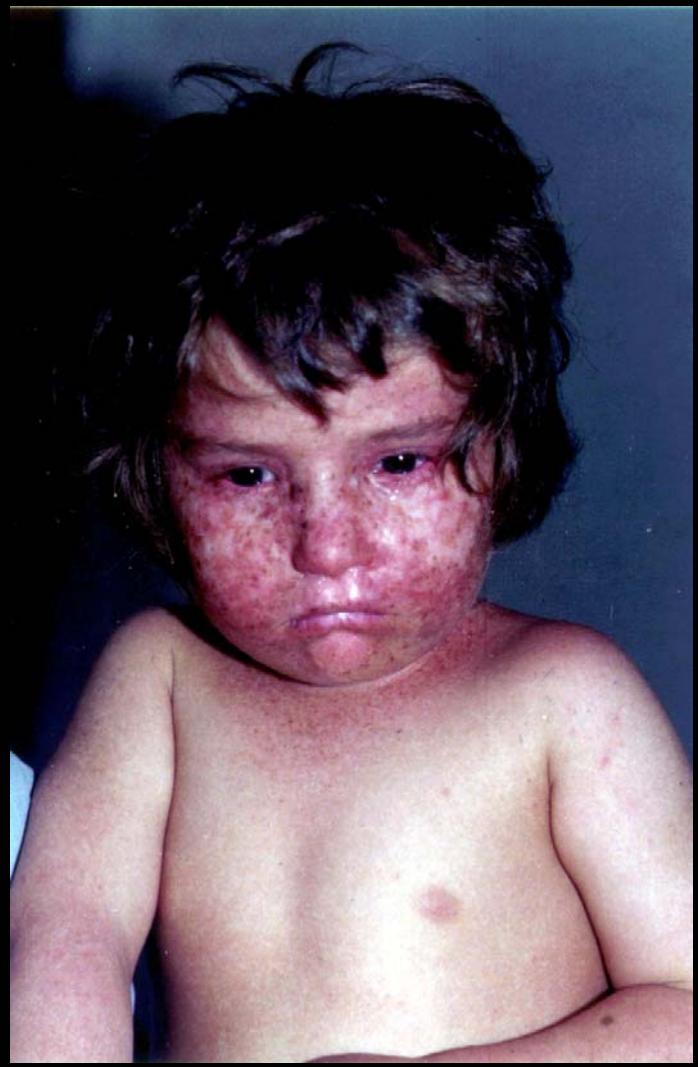
- known function

- very high cancer risk

TURKISH XP PATIENTS



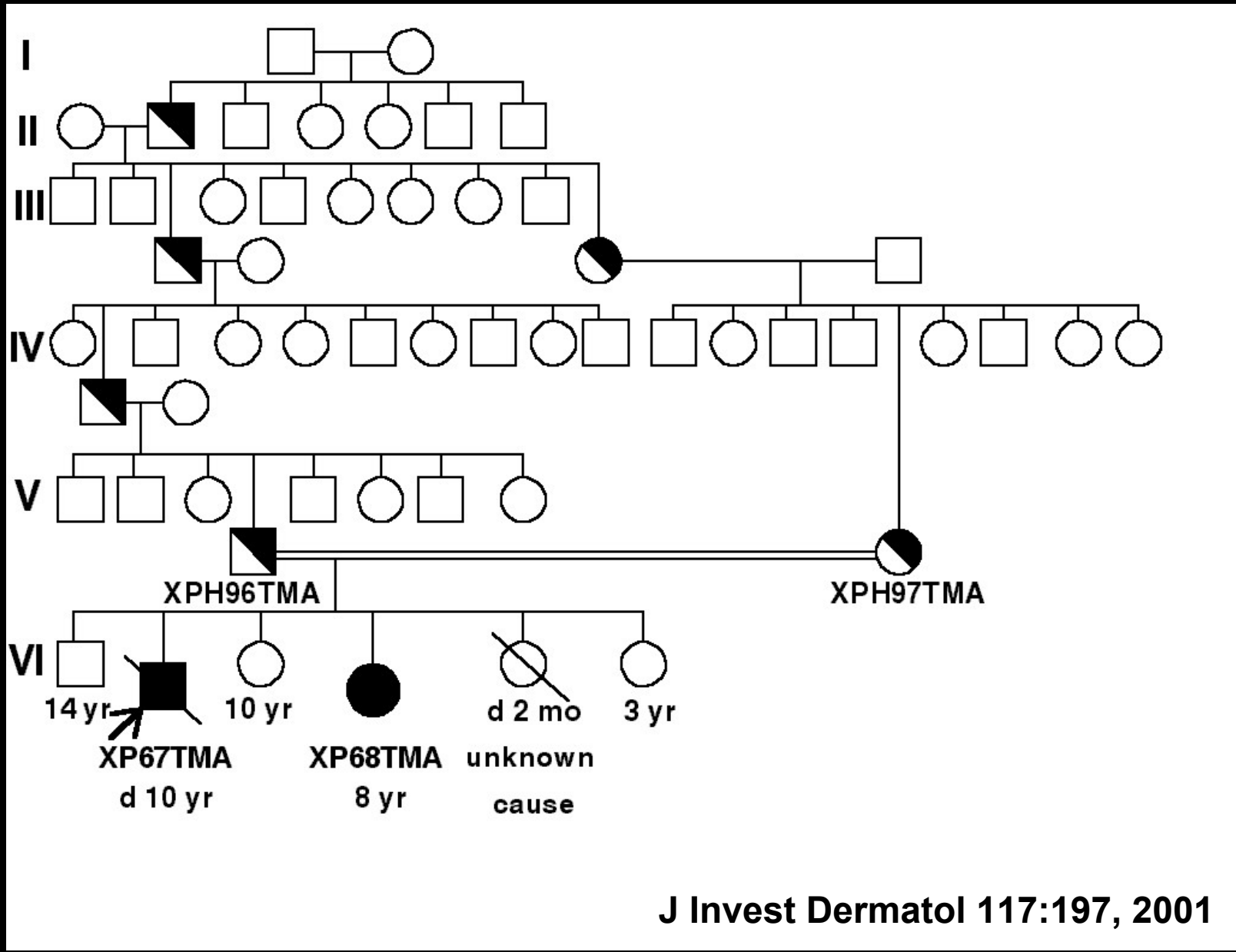
XP67TMA 7 y/o



XP68TMA 5 y/o

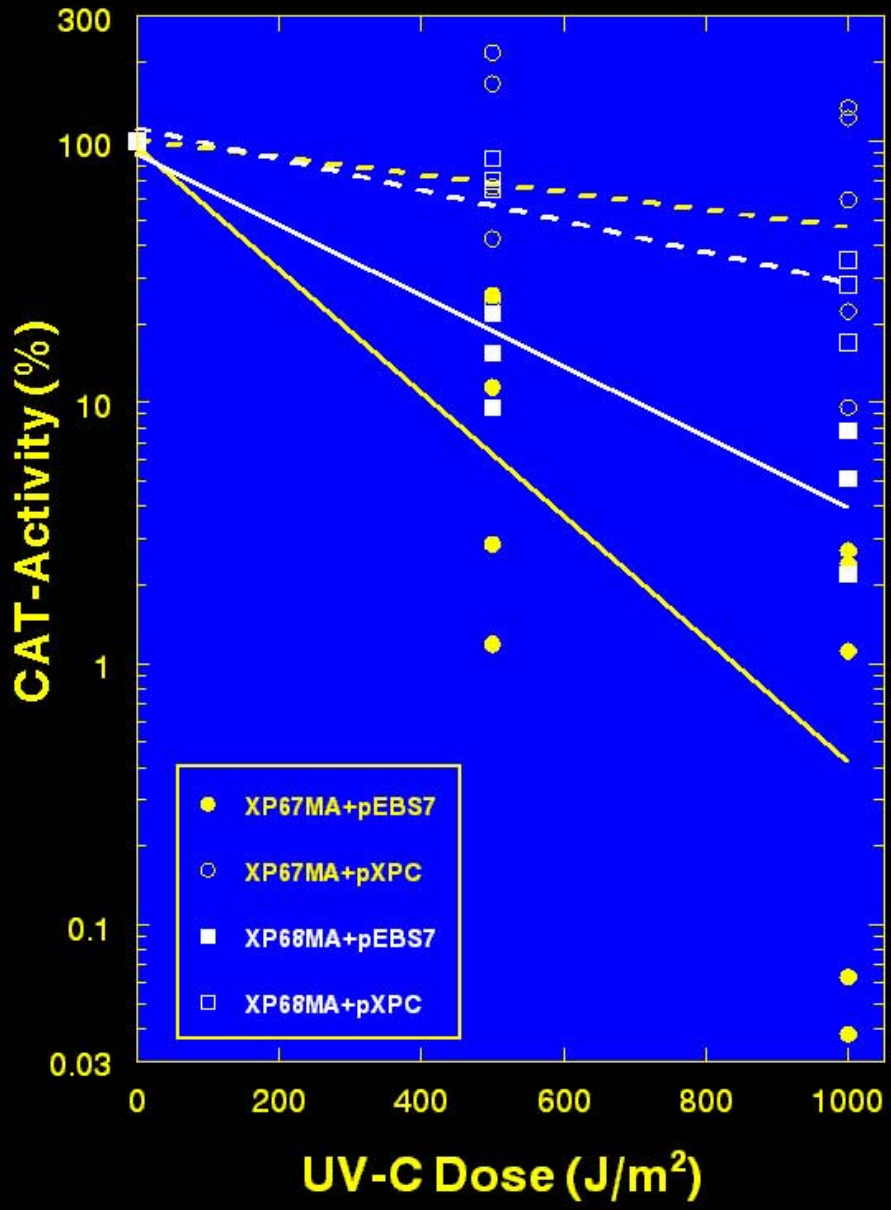
J Invest Dermatol 117:197, 2001

XP FAMILY FROM VAN, TURKEY



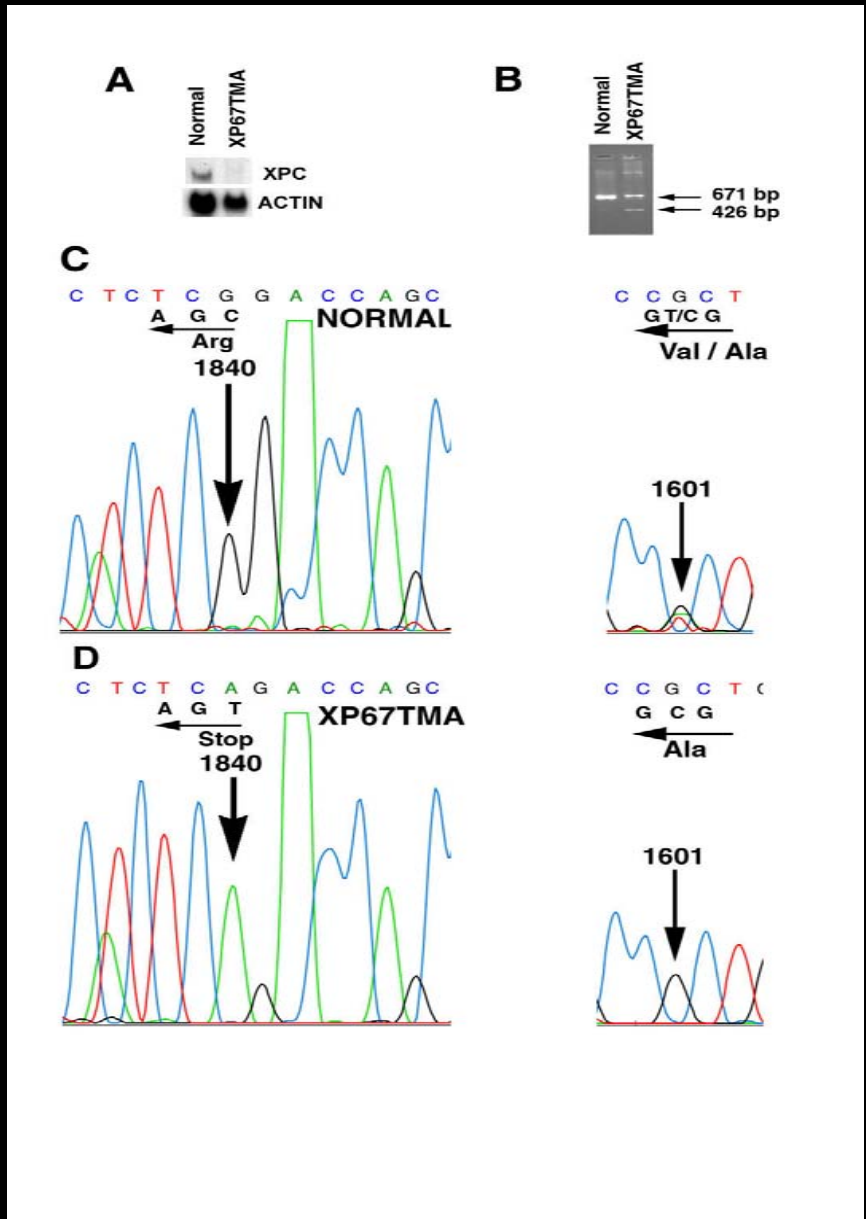
J Invest Dermatol 117:197, 2001

ASSIGNMENT OF XP67TMA and XP68TMA to XP COMPLEMENTATION GROUP C



JID 117:197, 2001

MOLECULAR ANALYSIS OF XPC cDNA IN XP67TMA



C1840T
Arg 579 Stop

J Invest Dermatol
117:197, 2001

GENETIC ANALYSIS OF XP-C FAMILIES IN TURKEY AND ITALY

Molecular genetic analysis of XPC alleles and flanking markers

Genethon	Microsatellite Marker	Turkish Alleles		Italian Alleles	
(cM)	Symbol	Paternal	Maternal	Paternal	Maternal
0	D3S1270(+)	5	5	5	4
1.4	D3S1307(+)	1	1	2	1
2.5	D3S1297(+)	1	4	3	4
	D3S1515(+)	1	3	2	1
16.5	D3S1304(+)	3	4	2	2
24.1	D3S1597	3	1	4	4
30.9	D3S1263	3	2	4	3
30.9	D3S1259	2	3	3	3
	M3-NT_022498-B14544	2	2	1	1
33	D3S1585	1	1	1	1
XPC	C1840T ARG579STOP	stop	stop	stop	stop
XPC	T1601C VAL499ALA	C	C	C	C
XPC	A2920C LYS939GLU	C	C	C	C
	M3-NT_005681-B2763	1	1	1	1
	M3-NT_005681-B84724	2	2	2	2
36.8	D3S3726	2	2	2	2
35.7	D3S1554	4	4	1	1
	M3-NT_005681-B20725	1	1	2	2
36.9	D3S1293	1	1	2	1
46.8	D3S1283	4	4	1	1
46.8	D3S1266	4	2	2	1
50.4	D3S3727	1	1	1	3

Large regions of identity indicate close (recent) relationships.

Smaller regions of identity indicate distant (older) relationships.

J Invest Dermatol 117:197, 2001

DETERMINATION OF GENETIC RELATIONSHIPS USING MICROSATELLITE MARKERS

$$R = 1 - e^{(-g\theta)}$$

(Luria and Delbruck, 1943) where

R is the proportion of chromosomes with recombination

g is the number of generations

θ is the genetic distance between recombined markers

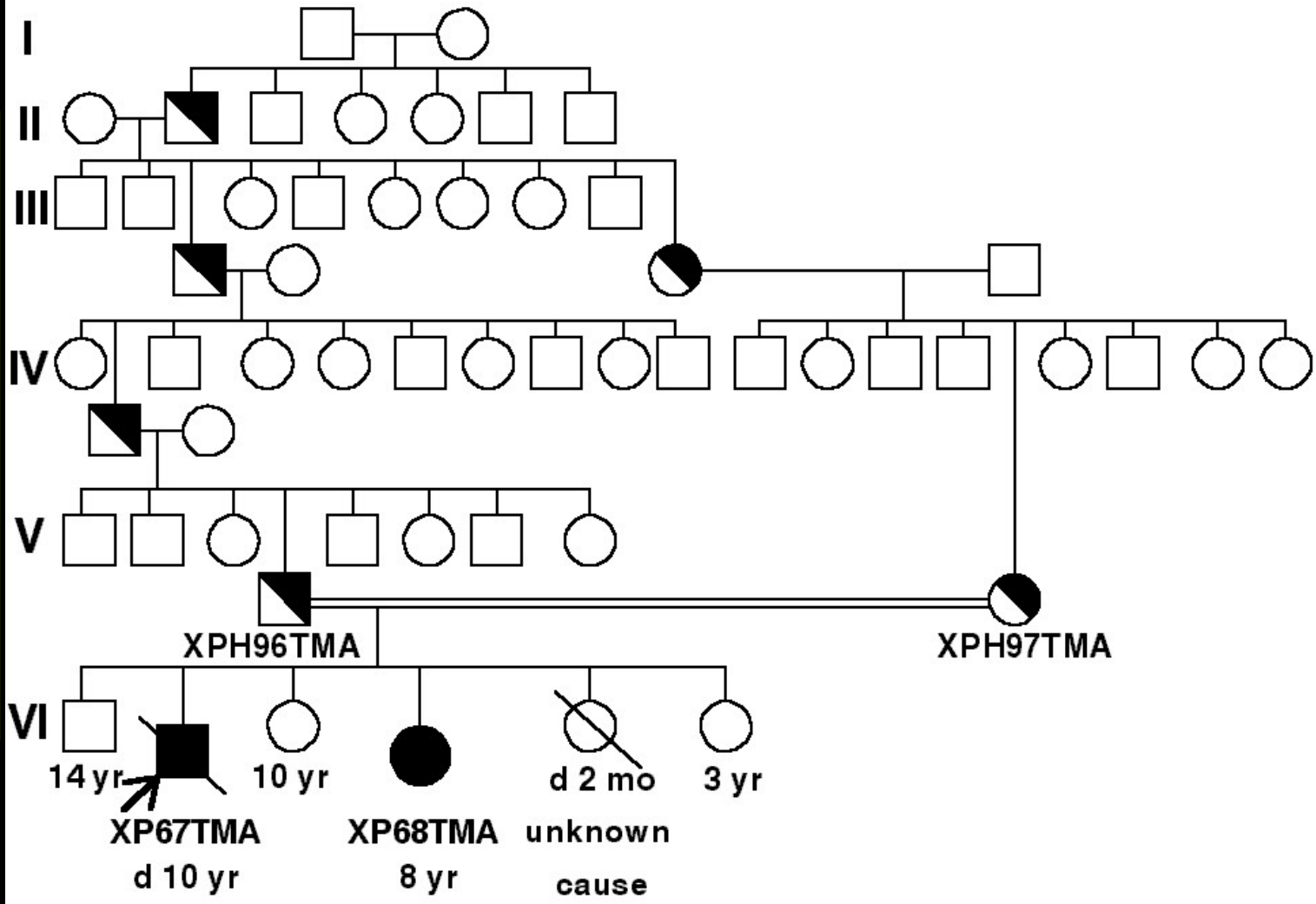
Turkish parents: R = 0.5 θ = 0.159 g = 4 generations

Italian parents: R = 0.5 θ = 0.06 g = 12 generations

J Invest Dermatol 117:197, 2001

XP FAMILY FROM VAN, TURKEY

4 generations



J Invest Dermatol 117:197, 2001

DETERMINATION OF GENETIC RELATIONSHIPS USING MICROSATELLITE MARKERS

$$R = 1 - e^{(-g\theta)}$$

(Luria and Delbruck, 1943) where

R is the proportion of chromosomes with recombination

g is the number of generations

θ is the genetic distance between recombined markers

Turkish parents: R = 0.5 θ = 0.159 g = 4 generations

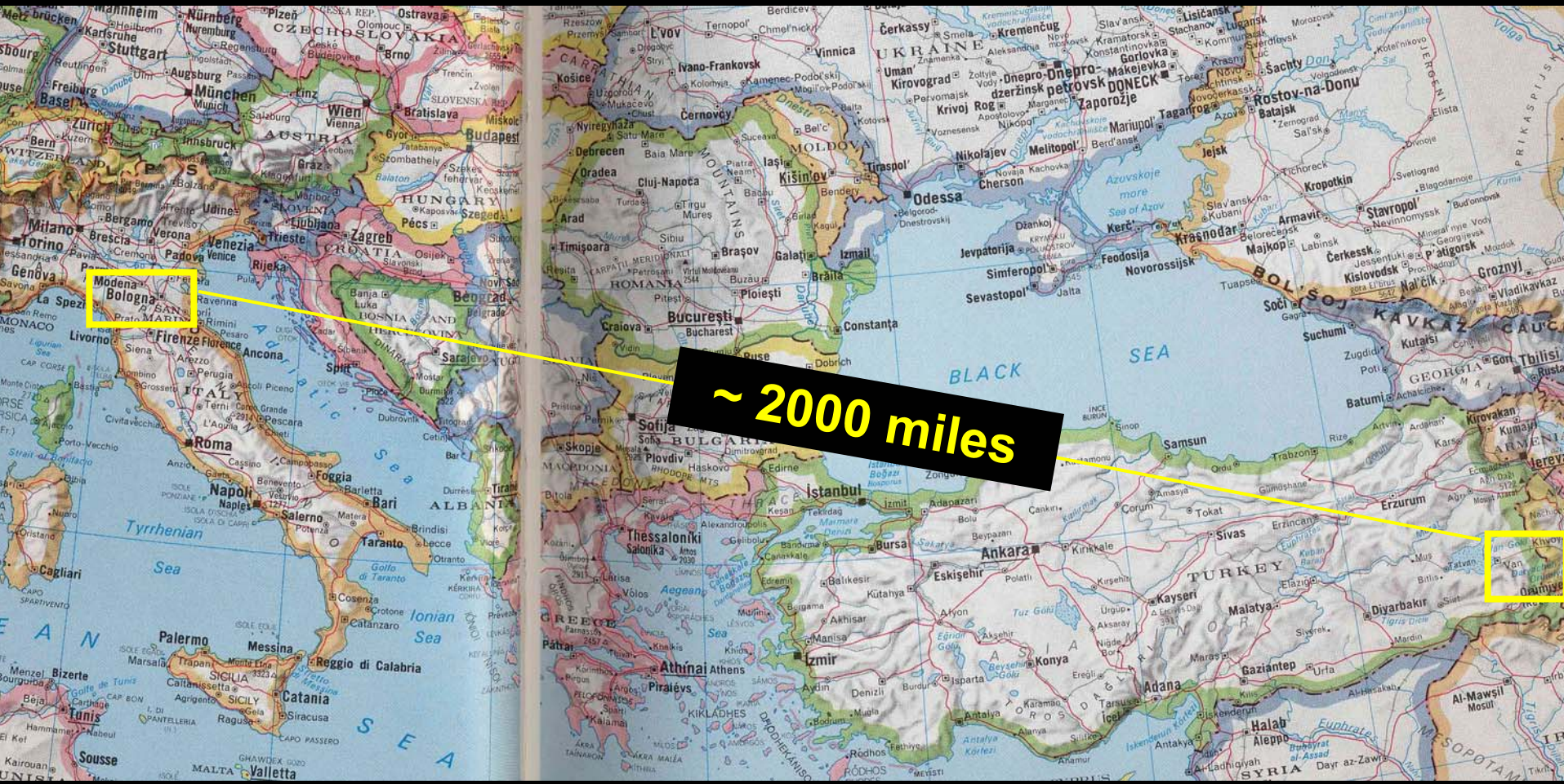
Italian parents: R = 0.5 θ = 0.06 g = 12 generations

Both families: R = 0.5 θ = 0.048 - 0.027 g = 14-26 generations

Assuming 1 generation is 20 years then this analysis suggests a common ancestor about 300-500 years ago.

J Invest Dermatol 117:197, 2001

XPC MUTATION MIGRATION BETWEEN BOLOGNA, ITALY and VAN, TURKEY 300-500 years ago



DNA REPAIR GENES AND CANCER RISK

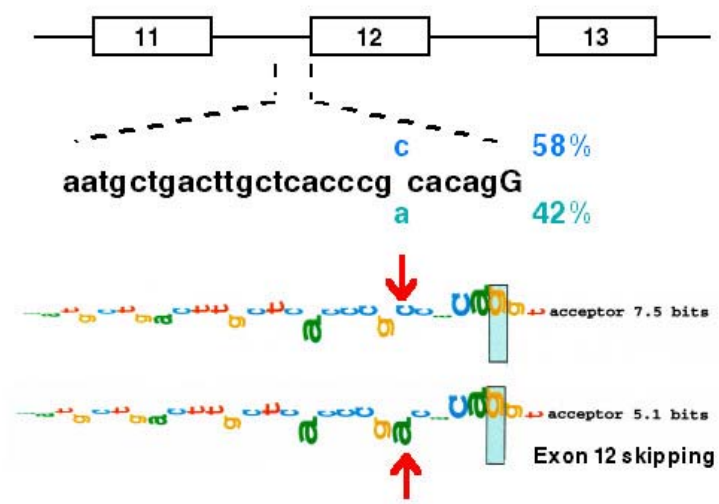
Disease gene homozygotes

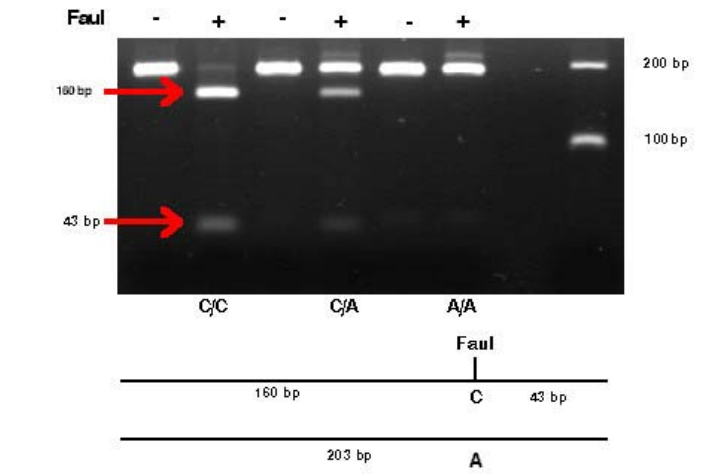
- low frequency
- known function
- very high cancer risk

Polymorphisms in the general population

- frequency > 1%
- unknown function
- unknown cancer risk

XPC SPLICE ACCEPTOR POLYMORPHISM

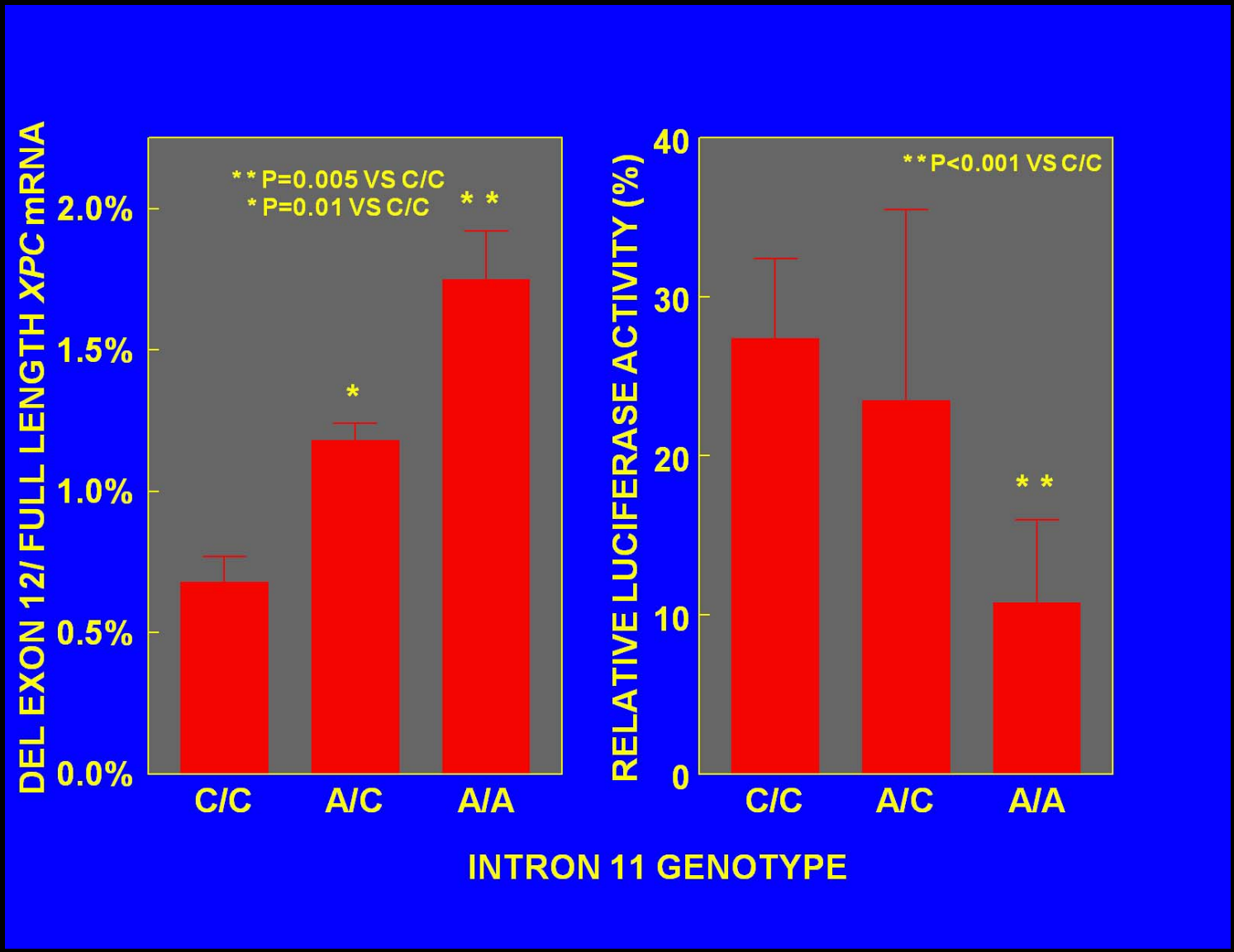




	NIH donors	Genotype distribution observed		
		C/C	C/A	A/A
		p^2	2pq	q^2
Number	97	37	38	22
Frequency	100%	38%	39%	23%

Nucleic Acids Research 30: 3624, 2002

RELATIONSHIP OF *XPC* INTRON 11 GENOTYPE TO ABNORMAL SPLICING AND REDUCED DNA REPAIR FUNCTION



Nucleic Acids Research 30: 3624, 2002

XPC PAT+ ALLELE IS A MARKER OF INCREASED RISK OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

	CASES	CONTROLS	P VALUE
TOTAL NUMBER	287	311	
PAT + ALLELE FREQUENCY	0.409	0.333	0.007

	NUMBER (%)			ADJUSTED ODDS RATIO (95% CI)			TREND TEST
PAT GENOTYPE	-/-	+/-	+/+	-/-	+/-	+/+	P
CASES	102 (35.6)	135 (47.0)	50 (17.4)	1.00	1.44 (1.01-2.05)	1.85 (1.12-3.05)	0.007
CONTROLS	141 (45.3)	133 (42.8)	37 (11.9)				

Cancer Res. 61:3321 (2001)

DNA REPAIR GENES AND CANCER RISK

Disease gene homozygotes

- low frequency
- known function
- very high cancer risk

Polymorphisms in the general population

- frequency > 1%
- unknown function
- unknown cancer risk

Disease gene heterozygote

- intermediate frequency
- known function
- unknown cancer risk

XP HETEROZYGOTES ARE MUCH MORE FREQUENT THAN HOMOZYGOTES

Hardy Weinberg equilibrium

$X^2 + 2Xy + y^2$

In US:

XP DISEASE FREQ (y^2) = ABOUT 10^{-6}

then $y = 10^{-3}$

NORMAL (X^2) = 1- y^2 or about 1

Heterozygotes (2Xy) = 2/1000 or 1/500

DO XP HETEROZYGOTES HAVE INCREASED CANCER RISK?

- Swift, M and Chase, C. Cancer in Families with Xeroderma Pigmentosum JNCI 62:1415,1979
- Studied 31 families - 2597 blood relatives and spouse controls
- Nonmelanoma skin cancer: 30/1046 blood rel vs 11/855 spouses p=0.02 OR 2.3 [1.1-4.5]
- Largest effect in 4 families: 20/219 rel vs 1/164 spouses p=0.0001 OR 16 [2.2-123]
- This study was before XP genes cloned thus no lab assay for confirmation XP genotype

CANCER RISK IN XP HETEROZYGOTE MICE

- *XPA* or *XPC* homozygous knockout mice have increased UV cancer susceptibility

- 1995 Sands et al: No increased post-UV skin cancer in *XPC* heterozygous mice after short exposure time

- 2000 Cheo et al: *XPC* heterozygous mice had increased post-UV skin cancer frequency after long exposure time (50 to 100 weeks)

PROPOSED STUDY TO EXAMINE CANCER RISK IN XP HETEROZYGOTES

Study of XP kindreds in US

1. Determine causative mutation in each kindred
2. Ascertain family members and determine cancer status
3. Determine presence or absence of causative mutation in DNA from family members using molecular diagnostic assays
4. Determine cancer frequency in XP heterozygotes and normal family members

XERODERMA PIGMENTOSUM FAMILIES STUDIED AT THE NIH

COMPLEMENTATION GROUP	NUMBER OF FAMILIES	NUMBER OF MUTATIONS
A	6	1
B	1	1
C	24	21
D	15	13
G	3	3
VARIANT	7	5
TOTAL	56	44


NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND, USA
NATIONAL CANCER INSTITUTE


NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND, USA
KRAEMER LAB
Sikandar Khan
Takahiro Ueda
Kyu Seon Oh
Kyoko Imoto
Hiroki Inui
Carine Nadem
NIH - CLINICAL
John DiGiovanna
Deborah Schmidt
Tom Hornyak – NCI
Jon Vogel - NCI
NIH – LAB
Carl Baker - NCI
Vilhelm Bohr - NIA
Tom Schneider – NCI
NIH – EPIDEMIOLOGY
Margaret Tucker - NCI
Alisa Goldstein – NCI
Kiyohiko Mabuchi – NCI
Roxana Moslehi - NCI
US
David Busch (deceased)
William Fishbein - AFIP
Qingyi Wei - M.D. Anderson
FOREIGN
Hanoch Slor - Israel
Engin Gozukara - Turkey
Shin-Ichi Moriwaki - Japan
Miria Stefanini - Italy
Koos Jaspers - Rotterdam
Jan Hoeijmakers - Rotterdam
Alan Lehmann – UK
Steffen Emmert – Germany